Large Lineage-Specific Expansions Are Driven By Mobile Genetic Elements

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ESPP Environmental Stress Pathway Project

DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT OFFICE OF SCIENCE U.S. DEPARTMENT OF ENERGY

VIMSS Virtual Institute for Microbial Stress and Survival

Massachusetts Institute of Technology

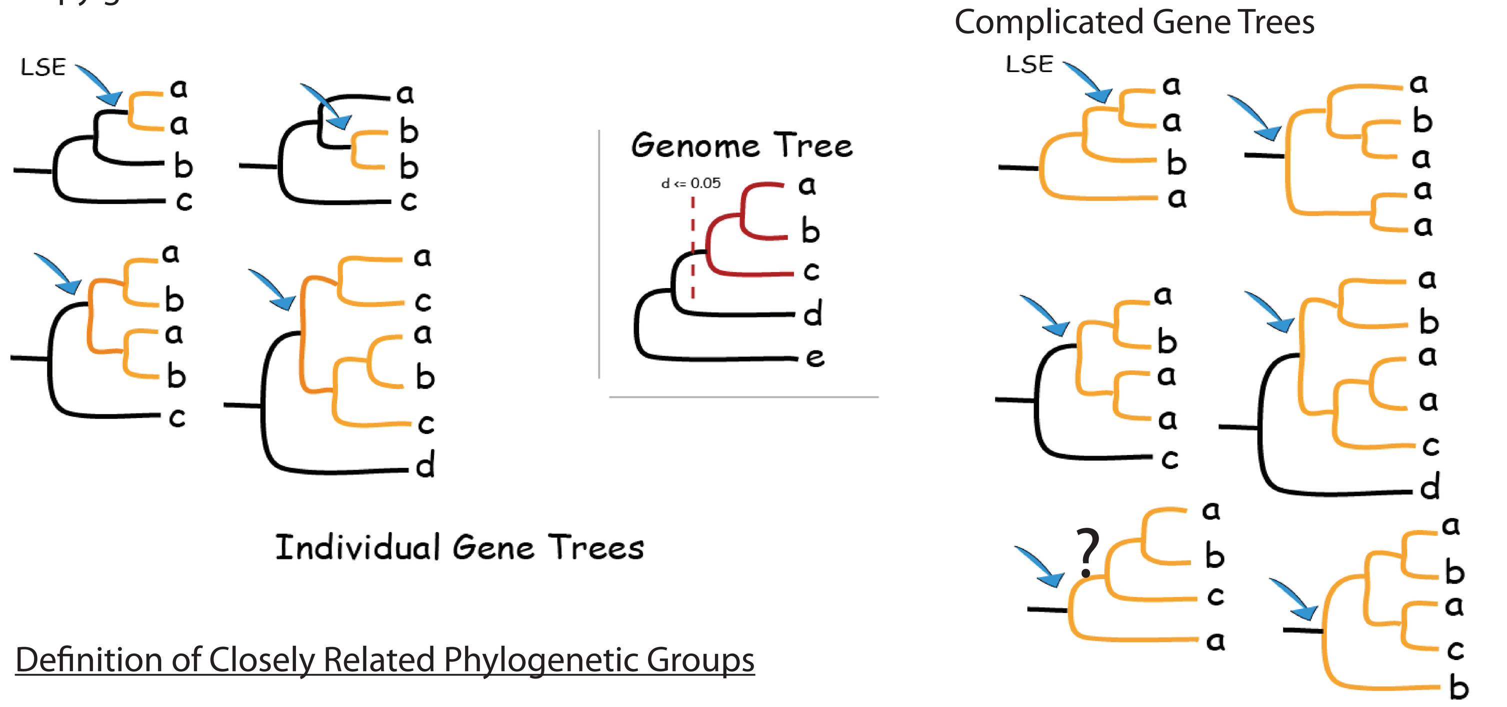
Abstract

Lineage-specific expansion (LSE) plays a vital role in how prokaryotes gain new gene functions and adapt to their environments. To uncover the mechanisms behind LSE, we identify genes that arise from LSE by constructing phylogenetic trees of protein families across 400+ bacterial genomes. We found that LSE genes tend to cluster on the chromosomes and form hyper LSE regions. Such regions could not be explained solely by operon duplication. The locations of these hyper LSE regions are often remarkably conserved among closely related strains, even though the gene content may not be conserved. Furthermore, these hyper LSE regions frequently overlap with clusters of mobile genetic elements (MGE) and strain-specific genomic islands. We hypothesize that the majority of large strain-specific gene duplications are mediated by MGE and are concentrated in regions prone to site-specific MGE-driven recombinations. And the same regions for the same reason are more susceptible to phage integration and to inter-genomic information exchange.

Methods

Identification of LSE

A LSE is identified by examining individual gene trees and finding the last common internal tree node among paralogous genes:
a) At least one gene duplication event is observed at the LSE node level.
b) To identify only recent events, all children of the LSE node must come from a single phylogenetic group of closely related species (see below).
c) Complicated gene trees require more sophisticated method to resolve, especially in cases when the LSE node includes genes from multiple genomes, but some of these genomes are present as single-copy genes.



Definition of Closely Related Phylogenetic Groups

Closely related genomes are grouped if pair-wise divergence is less than 5% (or d<=0.05) based on a Maximum-Likelihood tree constructed from a concatenated multiple sequence alignment of 70 highly conserved proteins.

Genome list at d<=0.05 in *E. coli* K12 group

group ID

855	Erwinia carotovora subsp. atroseptica SCRI1043
855	Escherichia coli 536
855	Escherichia coli APEC O1
855	Escherichia coli CFT073
855	Escherichia coli K12
855	Escherichia coli O157:H7
855	Escherichia coli O157:H7 EDL933
855	Escherichia coli UTI89
855	Escherichia coli W3110
855	Photorhabdus luminescens subsp. laumondii TTO1
855	Salmonella enterica subsp. enterica serovar Choleraesuis str. SC-B67
855	Salmonella enterica subsp. enterica serovar Paratyphi A str. ATCC 9150
855	Salmonella enterica subsp. enterica serovar Typhi
855	Salmonella enterica subsp. enterica serovar Typhi Ty2
855	Salmonella typhimurium LT2
855	Shigella boydii Sb227
855	Shigella dysenteriae Sd197
855	Shigella flexneri 2a str. 2457T
855	Shigella flexneri 2a str. 301
855	Shigella flexneri 5 str. 8401
855	Shigella sonnei Ss046
855	Yersinia pestis Antiqua
855	Yersinia pestis biovar Medievalis str. 91001
855	Yersinia pestis CO92
855	Yersinia pestis KIM
855	Yersinia pestis Nepal516
855	Yersinia pseudotuberculosis IP 3295

By grouping organisms at d<=0.05, the *Bacillus* genus are separated into 4 groups.

284	Bacillus anthracis str. 'Ames Ancestor'
284	Bacillus anthracis str. Ames
284	Bacillus anthracis str. Sterne
284	Bacillus cereus ATCC 10987
284	Bacillus cereus ATCC 14579
284	Bacillus cereus ZK
284	Bacillus thuringiensis serovar konkukian str. 97-27
288	Bacillus licheniformis DSM 13
288	Bacillus subtilis
291	Bacillus halodurans
292	Bacillus clausii KSM-K16

Terminology

LSE: the last common internal tree node among paralogs observed at d<=0.05. In this study, it refers to the genomic full-length LSE unless noted otherwise.
LSE size: number of genes in LSE normalized by number of genomes observed in the LSE node.
LSE genes: genes from the same LSE node.

Types of LSE

MGE LSE: LSE that includes mobile genetic elements, such as transposases, IS elements, integrases, etc.
Prophage LSE: LSE that includes prophage genes that are not MGE.
Phage-like LSE: LSE that includes other phage homologs that are not prophage LSE nor MGE.
Plasmid LSE: LSE that includes plasmid genes that are not in the above categories.
Domain LSE: LSE that includes chromosomal genes that are not in the above types and of which duplication occurs at the domain level.
Full-length LSE: the rest of the chromosomal LSEs, of which duplication occurs at the full-length protein

Results

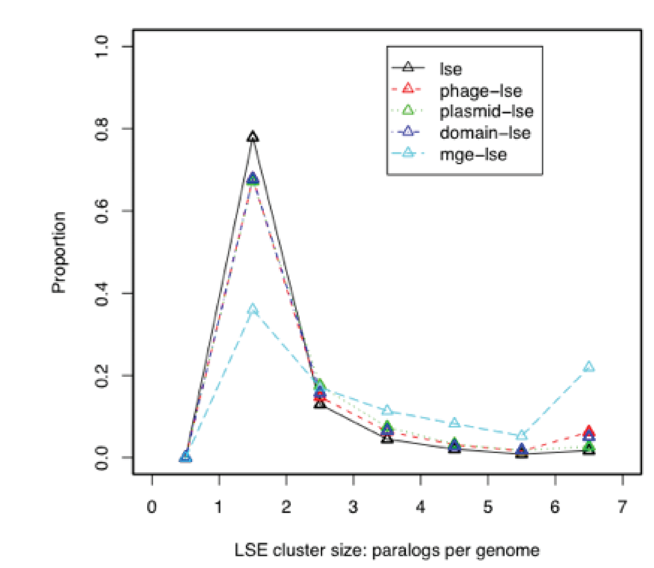
Overview of LSE

Summary of all identified LSE by categories.

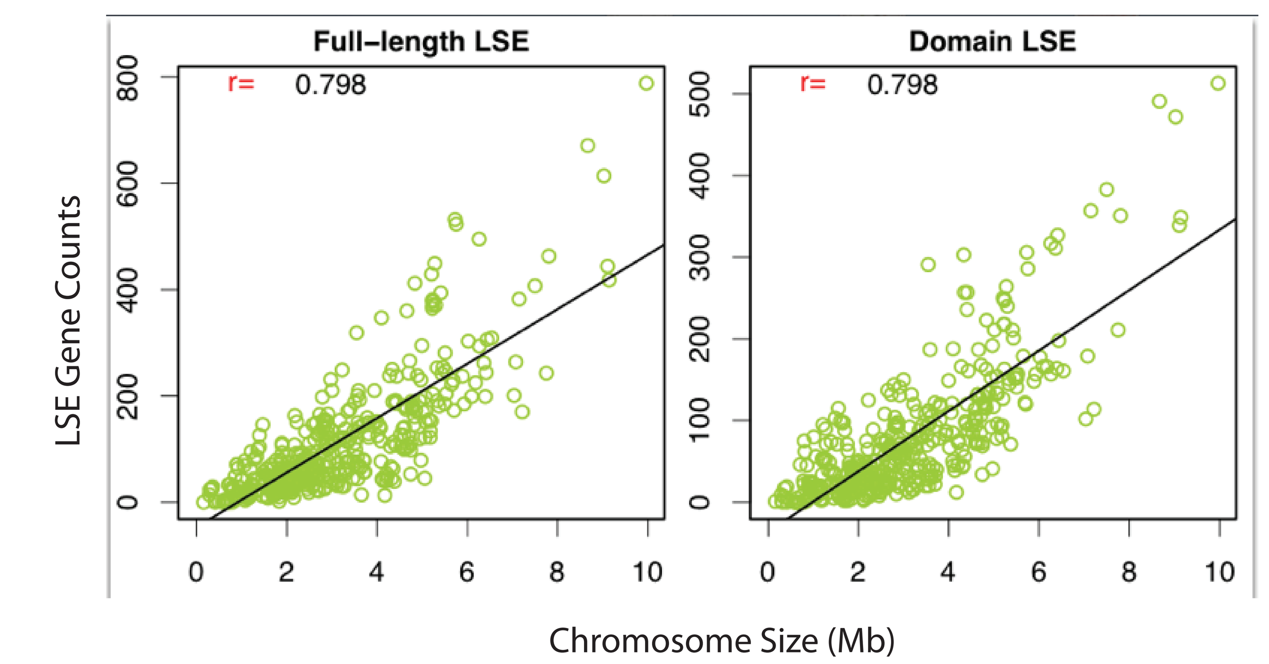
	LSE Genes	LSE	Genes per LSE	Genomes per LSE	LSE size (genes/genomes)
MGE	22,809	2,137	10.67	1.86	5.74
Prophage	9,063	892	10.16	4.19	2.43
phage-like	5,920	903	6.56	2.15	3.05
Plasmid	9,718	1,938	5.01	1.80	2.78
Genomic Domain	32,457	5,738	5.66	2.12	2.67
Genomic Full-length*	**46,661**	**10,491**	**4.45**	**2.09**	**2.13**
Total	126,628	22,099	N/A	N/A	N/A

* about 1500 FL-LSEs present only once in some genomes.

The majority of LSEs are small expansions



Abundance of LSE genes in genomes is correlated with genome size



LSE vs Operons

Is on polycystronic operon?

Is LSE gene ?

Escherichia coli K12

	No	Yes
Yes	224	296
No	1500	2377

P = 0.056

Bacillus subtilis

	No	Yes
Yes	127	101
No	1700	2273

P = 0.00015

Desulfovibrio vulgaris Hildenborough

	No	Yes
Yes	76	103
No	1347	2065

P = 0.056

Streptomyces coelicolor A3(2)

	No	Yes
Yes	987	646
No	3761	2674

P = 0.15

Prochlorococcus marinus MED4

	No	Yes
Yes	69	16
No	1022	873

P < 1e-06

Methanosarcina mazei Goe1

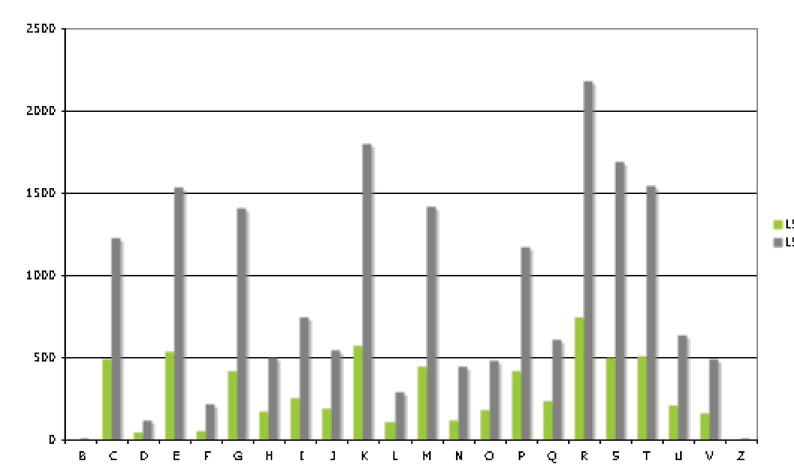
	No	Yes
Yes	425	388
No	1093	1419

P < 0.0001

*Genes that are MGE are excluded in this test

There are many examples of operon duplication. However, based on this simple Fisher Exact test, LSE genes either tend to be single-gene transcripts (p<0.01) or do not have a significant preference comparing to non-LSE genes.

COG functions in LSE



E: Amino acid transport and metabolism
K: Transcription
R: General function prediction only
S: Function unknown
T: Signal transduction mechanisms

Large LSEs tend not to have COG

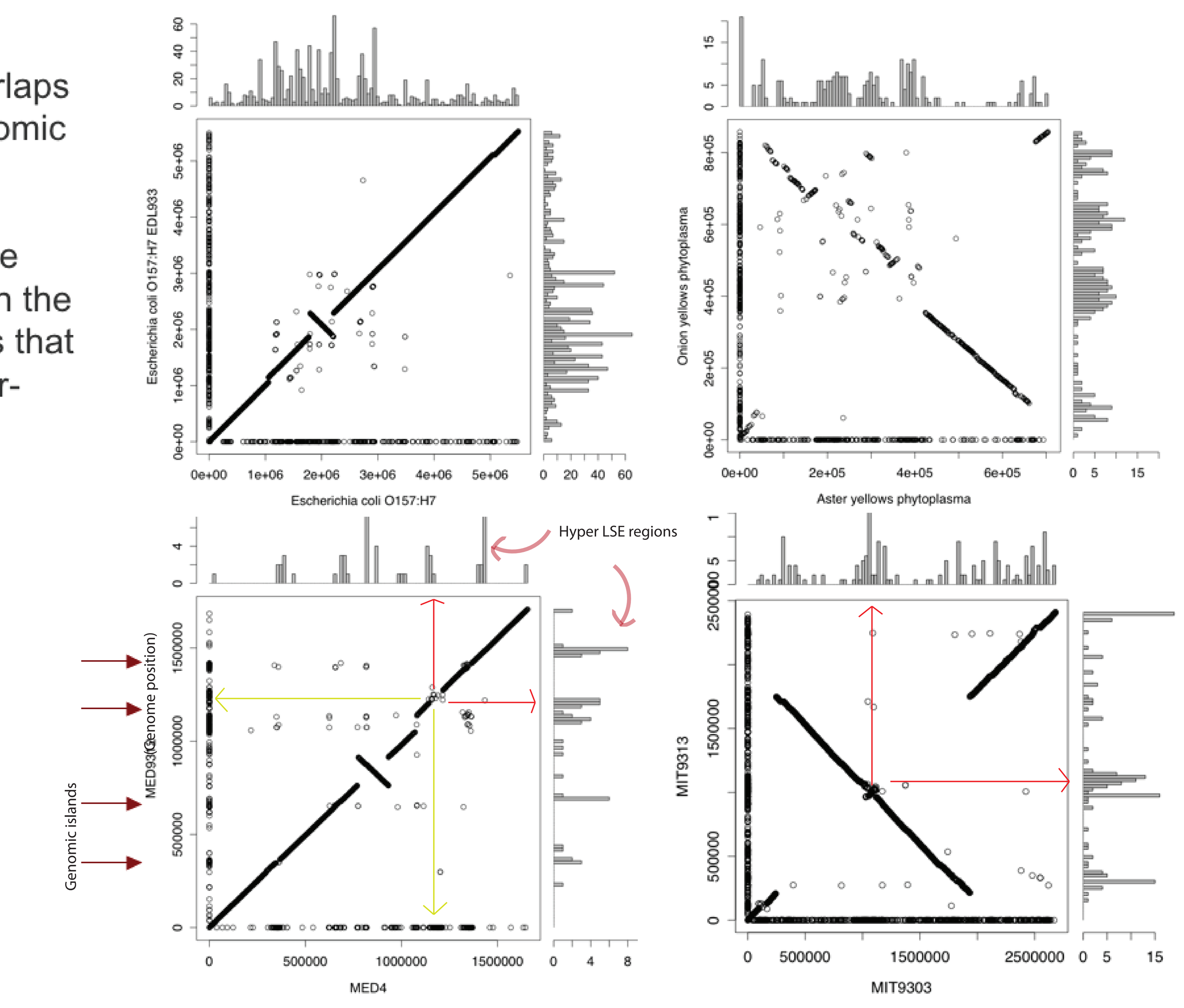
	LSE size>2	LSE size=2
has cog	798	4901
no cog	896	2024

p<1e-35

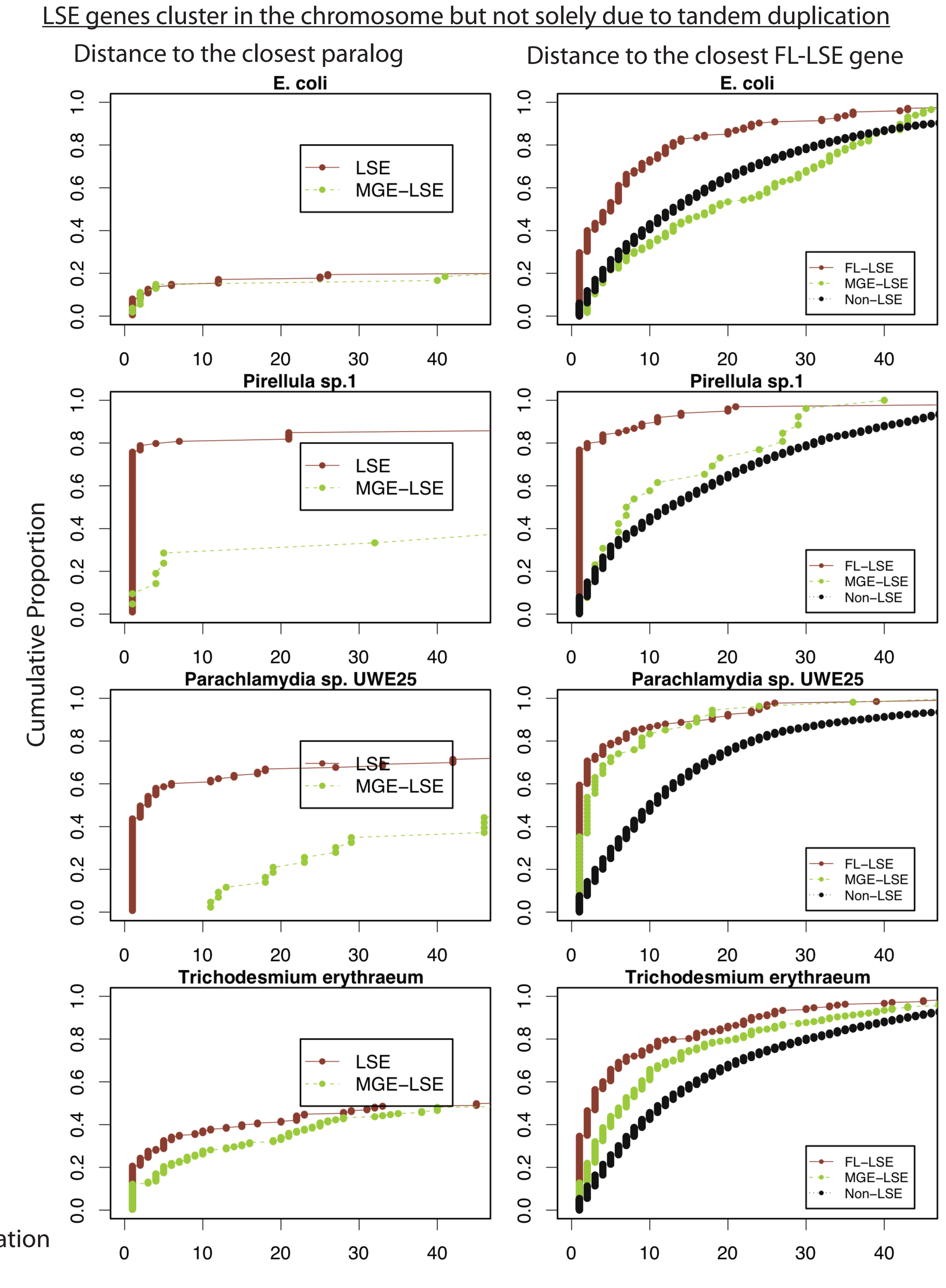
Pair-wise genome alignments reveal LSE hot spots

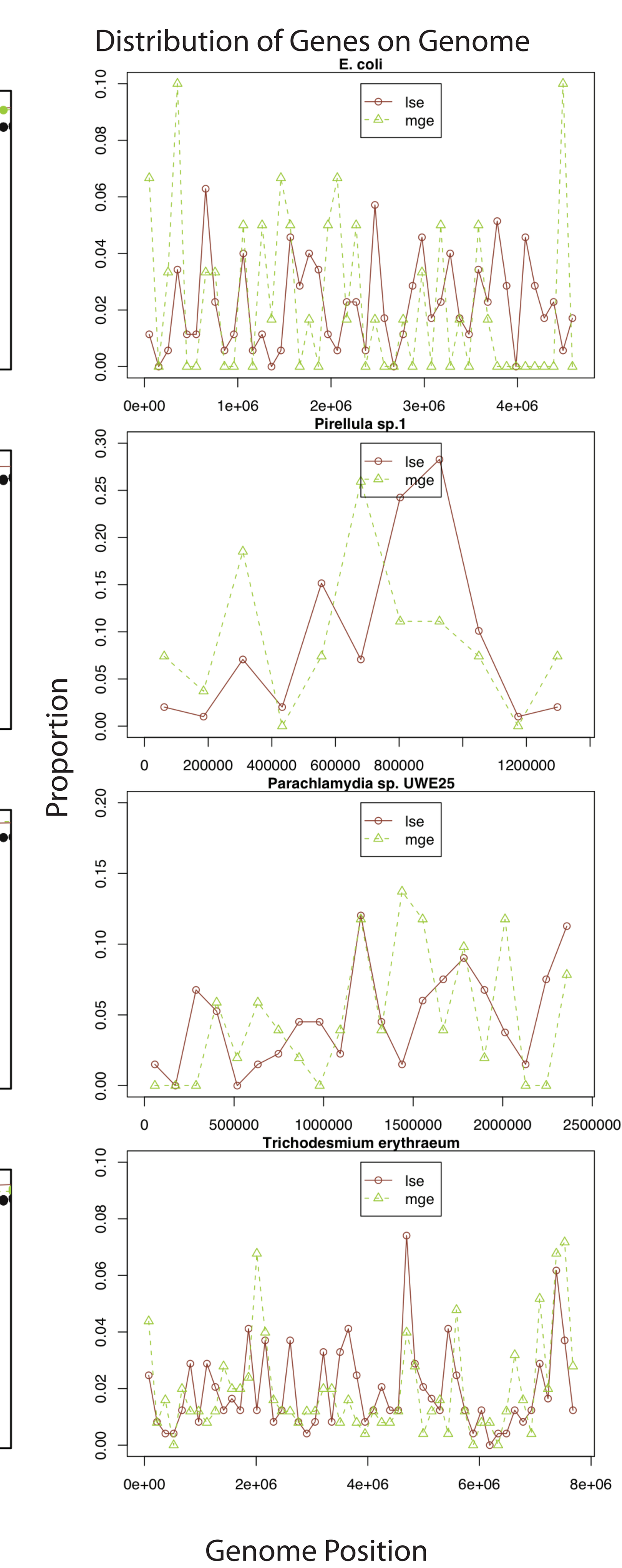
LSE overlaps with genomic islands.

These are regions in the genomes that are hyper-variable.

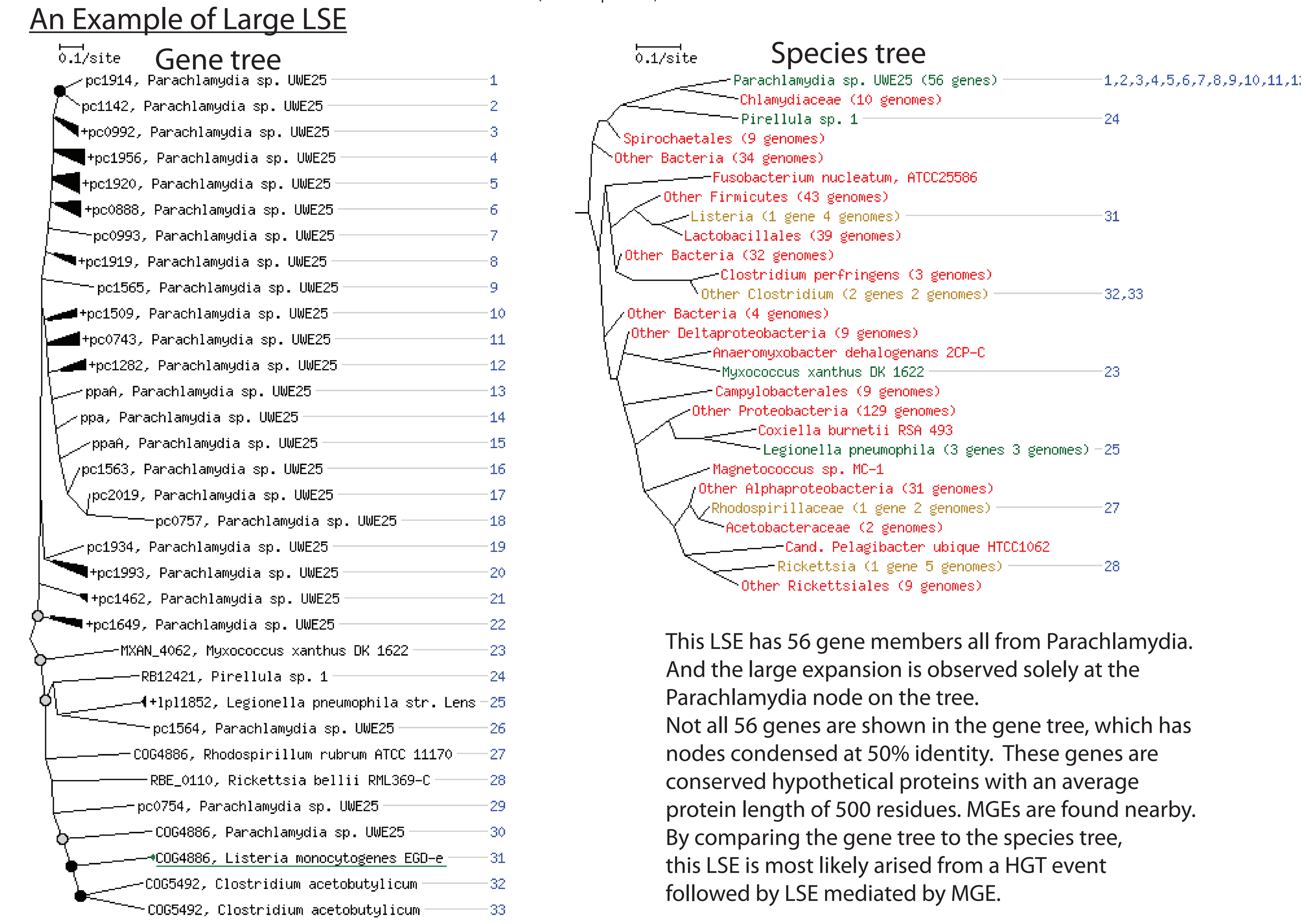


LSE genes cluster in the chromosome but not solely due to tandem duplication





An Example of Large LSE



This LSE has 56 gene members all from Parachlamydia. And the large expansion is observed solely at the Parachlamydia node on the tree.
Not all 56 genes are shown in the gene tree, which has nodes condensed at 50% identity. These genes are conserved hypothetical proteins with an average protein length of 500 residues. MGEs are found nearby. By comparing the gene tree to the species tree, this LSE is most likely arised from a HGT event followed by LSE mediated by MGE.

Conclusions

In this comparative genomic survey, we examine the types, functions, evolutionary history and mechanisms of LSEs. We have found that large LSE events are rare, out of more than 10,000 FL-LSE, only 16 of them have a size>15 genes. Although a couple COG functions are enriched in LSEs, a more significant observation is that larger LSEs (size>2) tend not to have COG assignments and over 60% of them are orphan genes or arised from recent HGT. In addition, many LSE genes seem to co-localize with MGEs or phage genes, especially those with a large size although more statistical tests are necessary to confirm this. We hypothesize that large LSEs are driven by MGEs or are uncharacterized MGEs themselves.

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